HEADQUARTERS
U.S. ARMY ENVIRONMENTAL HYGIENE AGENCY
UNITED STATES ARMY MEDICAL SERVICE

CLASS II ACTIVITY
OF
THE SURGEON GENERAL

EDGEWOOD ARSENAL
MARYLAND

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IN REPLY REFER TO
USAEHA-MT

PRIMARY IRRITATION EVALUATION PROGRAM ANNUAL REPORT 1963-64

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1. PURPOSE. Studies were conducted to classify chemical compounds according to primary skin- and eye-irritant effects on albino rabbits so that development research can proceed without undue hazard to personnel.

2. PROCEDURES.

a. Chemicals received by USAEHA for toxicologic evaluation are tested for primary skin irritation according to the method described in paragraph 3a. From results of the tests, the respective chemicals are classified according to established "Primary Skin Irritation Categories" /see subparagraph 3a(3)/.

b. All compounds which produce no skin irritation (Category I) and other compounds of special interest are tested further for injury to the eyes according to the method described in paragraph 3b, and the chemicals are classified according to established "Eye Injury Categories" /see subparagraph 3b(3)/. Results of PIEP testing are submitted in report form to the Office of The Surgeon General for consideration prior to transmittal to the activities concerned.

3. TESTING.

a. Primary Skin Irritation Patch Test.

(1) Method. The patch test technique is a modification of that described in Draize, et al[1] and Ambrose[2]. At least four albino rabbits are used for each test agent. The intact and abraded skin on the back of the rabbit is used. Four areas of the back (see Figure 1) are designated for the patches, which consist of 1" by 3" adhesive plastic strips. Areas 2 and 3 are abraded by scratching the skin of the test animals lightly with specially prepared abraders. One-half

milliliter of the material to be tested is introduced in liquid form under the patch. Solid materials are dissolved in a suitable solvent that has been previously tested for nonirritability--usually ethyl alcohol. The volume applied under the patch contains 0.5 gram of the solid material. The animals are wrapped with elastic bandages and strips of muslin before they are returned to their cages. The materials are allowed to remain on the animals for 24 hours.

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Rabbit 1		Rabbit 2		Rabbit 3		Rabbit 4	
1	2*	1	2*	1	2*	1	2*
3*	4	3*	4	3*	4	3*	4

Figure 1
*Abraded Skin

If an animal dies during the test, and the cause of death cannot be determined by autopsy or other means, a second test is made to determine whether death was caused by any of the test agents. At least one milliliter (preferably two) of the test agents is applied under a gauze patch to the back of each of the four rabbits, and allowed to remain for four days. If all the animals survive, the conclusion is that the death was not caused by the test material.

(2) Scoring. Twenty-four hours fter application, the patches are removed and the reactions produced by the compounds are evaluated on the basis of weighted scores (Table 1). A second evaluation is made at the end of 72 hours and the combined scores are used for final classification. The animals are observed for 14 days after the start of the test for possible delayed systemic or local effects.

Table 1

Evaluation of Skin Reactions

A. Erythema and Eschar Formation	
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate-to-severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema	4

B. Edema Formation

Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (edges raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Total possible edema score	4
Total possible score for primary irritation	8

(NOTE: Any skin reaction greater than severe edema or erythema is cause for a chemical's being classified in Category V or VI.)

(3) Primary Skin Irritation Categories. To facilitate reporting, category symbols are used in conjunction with definitions indicating the relative order of the skin-irritating properties of the chemicals tested. The categories are as follows:

Category I - Compounds producing no primary irritation of the intact skin or of the skin surrounding an abrasion.
Primary irritation score limits: (Intact and abraded) 0 - 0.5
*Interpretation - There is no restriction for acute application to the human skin.

Category II - Compounds producing no primary irritation of the intact skin, but producing mild primary irritation of the skin surrounding an abrasion.
Primary irritation score limits: (Intact) 0.0 - 0.5 (Abraded) 0.51 - 2.0
*Interpretation - Compounds to be used only on human skin found by examination to have no abrasions or to be used as a clothing impregnant.

Category III - Compounds producing mild primary irritation of the intact skin, and of the skin surrounding an abrasion.
Primary irritation score limits: 0.51 - 2.0
*Interpretation - Compounds to be used as clothing impregnants only.

* Interpretations of results are intended only for guidance in conducting further biologic testing of the compounds as personal insect control chemicals.

Category IV - Compounds producing moderate primary irritation of the intact skin and other skin surrounding an abrasion.
Primary irritation score limits: 2.1 - 5.0
*Interpretation - These compounds should not be used on the skin or as clothing impregnants, except with extreme caution.

Category V - Compounds producing moderate primary irritation of the intact skin and of the skin surrounding an abrasion; and, in addition, producing necrosis, vesication, ulceration, and/or eschars.
Primary irritation score limits: 2.1 - 5.0
*Interpretation - These compounds should not be used on the skin or as clothing impregnants.

Category VI - Compounds producing severe primary irritation of the intact skin and of the skin surrounding an abrasion; and, in addition, producing necrosis, vesication, ulceration, and/or eschars.
Primary irritation score limits: 5.1 - 8.0
*Interpretation - These compounds should not be used on the skin or as clothing impregnants.

Category VII - Compounds not possible to classify because of staining of the skin or because of other masking effects due to physical properties.
*Interpretation - Not suitable for use on human skin.

b. Preliminary Eye Injury Test.

(1) Method.

(a) Procedures are derived from the methods of Draize, et al[1], Ambrose[2], and Carpenter and Smyth[3]. Chemicals tested are those which did not produce any signs of skin irritation (Category I).

(b) Healthy adult albino rabbits are selected on the basis of absence of extensive grossly visible lesions of the eye, by staining with a 5-percent aqueous solution of sodium fluorescein, flushing with warm distilled water one minute after application, and observing the eye under an ultraviolet light.

(c) A sketch is made of the rabbit's eyes showing the location and extent of any damage present. All early changes on the condition of the eye are marked on the sketch. Four to six hours after the stain is applied, the animals having no evident or interfering eye injury are considered to have returned to normal and are ready for the test.

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* Interpretations of results are intended only for guidance in conducting further biologic testing of the compounds as personal insect control chemicals.

(d) Two drops (approximately 0.2 ml) of the strongest possible concentration of the candidate solution are placed on the cornea which has been exposed by retracting the eyelids with the fingers. After one minute, the animal is released and returned to its cage. Each solution is administered to at least four eyes for the complete test. One eye of each rabbit is used with the test material, and the other eye is used as a control. Both eyes are examined each day before and after fluorescein staining and any changes noted are marked on the eye chart. Additional applications of the test solution are administered each day for five days, ten minutes after fluorescein staining, provided no extensive eye damage is noted. The injury, if any, is scored 24 hours later.

(2) Scoring.

(a) The eye injury produced by the candidate compound is evaluated according to the weighted scoring system used by Carpenter and Smyth[3] (Table 2).

Table 2

System for Numerical Scoring of Injury to the Rabbit's Eye Twenty-four Hours After Application of a Material

Effects Visible Before Fluroescein Staining	Points 100%	Points 50%	Maximum Total Points
Cornea dull	2	1	
Cornea opaque, less than half of the area	4		
Cornea opaque, more than half of the area	6		6
Keratoconus	6		6
Iritis, slight internal congestion	1		
Iritis, marked internal congestion	2		2

Effects Visible After Fluroescein Staining	Points 100%	Points 50%	Maximum Total Points
Necrosis on less than 5% of cornea	1		
Necrosis on 5 - 12%	2		
Necrosis on 13 - 37%	3		
Necrosis on 38 - 62%	4		
Necrosis on 63 - 87%	5		
Necrosis on 88 - 100%	6		6
(If necrosis is diffuse, assign points corresponding to half of the observed area)			
Total possible points			20

(b) The individual numerical scores of each eye to which a given compound is applied are added together and then divided by the number of eyes used to obtain the score of the injury caused by the compound. The injury to the conjunctiva is also noted in the record. Erythema, edema, and discharge from the eye are observed and noted.

(3) Eye Injury Categories. For convenience, the eye injury categories used are lettered and defined, and irritation score limits are given as follows:

Category A - Compounds, noninjurious to the eye.
Eye injury score limits: 0 - 0.5
*Interpretation - Unrestricted use.

Category B - Compounds producing mild injury to the cornea.
Eye injury score limits: 0.51 - 2.0
*Interpretation - To be used with caution around the eye.

Category C - Compounds producing mild injury to the cornea and, in addition, some injury to the conjunctiva.
Eye injury score limits: 0.51 - 2.0
*Interpretation - To be used with caution around the eye and mucosa.

Category D - Compounds producing moderate injury to the cornea.
Eye injury score limits: 2.1 - 5.0
*Interpretation - To be used with extreme caution. Keep away from ocular areas.

Category E - Compounds producing moderate injury to the cornea and, in addition, producing some injury to the conjunctiva.
Eye injury score limits: 2.1 - 5.0
*Interpretation - To be used with extreme caution. Keep away from ocular areas.

Category F - Compounds producing severe injury to the cornea and conjunctiva.
Eye injury score limits: 5.1 and over
*Interpretation - To be used with extreme caution. Recommend that use be restricted to areas other than face.

4. CLASSIFICATION. The following compounds are classified according to the "Definitions of Categories of Compounds".

ENT NO.	P.I.E.P. Skin	Category Eye			COMPOUND CHEMICAL NAMES
2706	I	F	(a)		2,2,4-Trimethyl-1,3-pentanediol
16634-i	VII	C	(b)		Sulfoxide-88% n-octyl sulfoxide of Isosafrole
17596-c	I	E	(c)		5a,6,9,9a,9b-Hexahydro-4a[4HO-dibenzolfurancarboxaldehyde (Phillips R11)]
20640	II	E			3-Hexanol, 3-methyl-6-phenyl-
20828	III				Malic acid, dl-, diisobutyl ester
20939	I	E			Veratrole, 4-propyl-
23451	I	B			Benzene, 2-(allyloxy)-1, 3-dimethoxy
23778	V		(d)		Acrylic acid, 2-cyano-3-ethoxy-, ethyl ester
23969-d	I	A	(e)		Sevin-1-napthyl-N-methylcarbamate
25671	I	A			o-Isopropoxyphenyl methylcarbamate
25720	III		(f)	†	Commercially discreet
25736a	I	A			2-Chloro-4,5-dimethyl phenyl methylcarbamate
25780	I	C			3,5 Diisopropylphenyl N-methylcarbamate (pure compound)
25810	I	A			o-Propargoxyphenyl-N-methylcarbamate
26337	V		(d)	†	Commercially discreet

† -Compounds submitted by private companies which have requested that the nomenclature by withheld from publication.

ENT NO.	P.I.E.P. Category Skin	Eye		COMPOUND CHEMICAL NAMES
26660-x	V		(d)	Octanamide, N,N-dimethyl-
27196	III	E		Morpholine, 4-(2-decemoyl)
32227	II	F		Quinoline, 1,2,3,4-tetrahydro-1-isobutyl
32490	I	A	(g,h)	Acetamide, N-butyl-2,2, dichloro-
32491	I	A	(g,h)	Acetamide, 2,2-dichloro-N-isobutyl-
32499	II	A	(g,h)	Acetamide, 2,2-dichloro-N-pentyl-
32640	I	F		Succinamic acid, N,N-diethyl, -methyl ester
32698	I	A		Benzoic acid, o-(4,5-dimethyl-1, 3-dioxolan-2-yl)-methyl ester
32705	II	C		m-Dioxane, 5-ethyl-2- (2-methoxyethyl)-4-propyl
32720	I	A		Phthalaldehydic acid, allyl ester
32722	II	C		Phthalaldehydic acid, propyl ester
32806	IV	C		Hexamethyleneamine, 1-chrysanthemmoyl
Mixture 23969-d 16634-i	VII	F	(i)	Sevin & sulfoxide -
None	I	A	(j)	GMF. Paraquinonedioxime
None	I	A	(k)	Irgasan (h); 5,6-dichlorobenz-oxazolinone-2. (powder)
None	I	F		Irgasan-dissolved in propylene glycol-

5. REMARKS.

a. Compound ENT 2706 was dissolved at the rate of 400 mg/ml of a nonirritating batch of propylene glycol for use in both the skin- and eye-irritation studies.

b. Sulfoxide ENT 16634 had a depilatory effect upon the hair in the anterior suborbital region of the female skin-test animals. Within two weeks of the final applications, the hair growth in both areas returned to normal.

c. Compound ENT 17596-c caused a slight green discoloration in the abraded skin-test area. Forty-eight hours after the initial application, eschar formation on the test area was observed.

d. Eschars were found at the site of application on the skin.

e. Sevin, ENT 23969 was dissolved in polyethylene glycol 400 at the rate of 20 mg/ml for use in the eye and skin tests.

f. ENT 25720 was mixed with cottonseed oil to form a suspension of 0.5-percent by weight; 1.0 gm of the paste was applied at the test site.

g. Compounds ENT 32420, 32491, 32499 were dissolved in ethyl alcohol in concentrations of 1 gm/ml, 0.57 gm/ml, and 1 gm/ml, respectively, for use on the skin.

h. For the eye tests, approximately 0.1 ml of a solution prepared by dissolving 0.2 gm of the agent in 3 ml of propylene glycol was placed in the conjunctival sac.

i. Sevin and sulfoxide were mixed in a ratio of 1:1 (one part 88-percent sulfoxide to one part 20 mg/ml Sevin in polyethylene glycol). Depilation by this mixture was not as severe as that produced by sulfoxide alone. No effect of the mixture was observed on the cornea, but the effect on the conjunctiva was severe.

j. Each day for five days 0.1 ml of a solution of GMF, 20 mg/ml in polyethylene glycol 400 was applied to the test eye and 0.5 ml one-half of the solution was applied to the skin.

k. Irgasan was prepared in a concentration of 83.3 mg/ml for use in the second eye-irritation test.

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